# MSK1 Kinase Assay

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## Scientific Background:

**MSK1 or mitogen- and stress-activated protein kinase-1 contains 2 protein kinase domains and shares 43% protein sequence identity with the MAPKAPK1 isoforms. Northern blot analysis shows that MSK1 is expressed in all tissues with the highest level of expression in brain, muscle, and placenta. Immunoelectron microscopy localized MSK1 to the nucleus. MSK1 is activated in vitro and in vivo by either ERK or SAPK2 proteins. MSK1 rather than MAPKAP-K1 or MAPKAP-K2/K3, mediates activation of the cAMP response element-binding protein and activating transcription factor-1 by either growth factors or stress signals. By radiation hybrid analysis, the RPS6KA5 gene has been mapped to chromosome 14q31-q32.**

1. Deak, M. et al: Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB. EMBO J. 17: 4426-4441, 1998.
2. Jiang, C. et al: Assignment of a member of the ribosomal protein S6 kinase family, RPS6KA5, to human chromosome 14q31-q32.1 by radiation hybrid mapping. Cytogenet. Cell Genet. 87: 261-261, 1999.

## ADP-Glo™ Kinase Assay

### Description

**ADP-Glo™ Kinase Assay is a luminescent kinase assay that measures ADP formed from a kinase reaction; ADP is converted into ATP, which is converted into light by Ultra-Glo™ Luciferase (Fig. 1). The luminescent signal positively correlates with ADP amount (Fig. 2) and kinase activity (Fig. 3A). The assay is well suited for measuring the effects chemical compounds have on the activity of a broad range of purified kinases—making it ideal for both primary screening as well as kinase selectivity profiling (Fig. 3B). The ADP-Glo™ Kinase Assay can be used to monitor the activity of virtually any ADP-generating enzyme (e.g., kinase or ATPase) using up to 1mM ATP.**

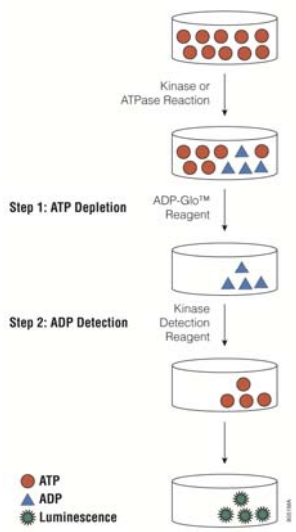



**Figure 1. Principle of the ADP-Glo™ Kinase Assay.** The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

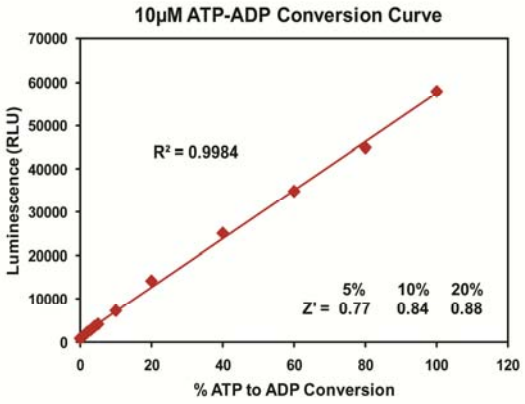



**Figure 2. Linearity of the ADP-Glo Kinase Assay.** ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

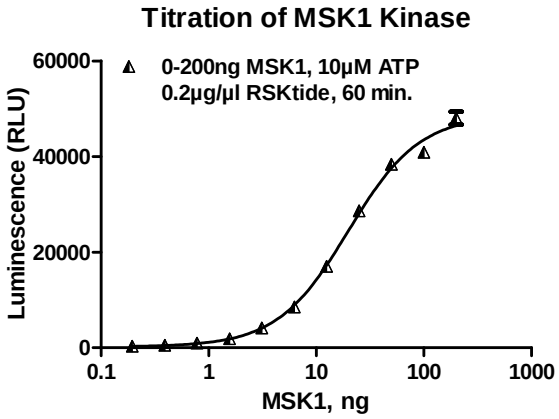
For detailed protocols on conversion curves, kinase assays and inhibitor screening, see *The ADP-Glo™ Kinase Assay* Technical Manual #TM313, and the KES Protocol available at: http://www.promega.com/tbs/tm313/tm313.html, and http://www.promega.com/KESProtocol, respectively.

## Protocol

- Dilute enzyme, substrate, ATP and inhibitors in Kinase Buffer.
- Add to the wells of 384 low volume plate:
  - 1 µl of inhibitor or (5% DMSO)
  - 2 µl of enzyme (defined from table 1)
  - 2 µl of substrate/ATP mix
- Incubate at room temperature for 60 minutes.
- Add 5 µl of ADP-Glo™ Reagent
- Incubate at room temperature for 40 minutes.
- Add 10 µl of Kinase Detection Reagent
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1sec).

**Table 1. MSK1 Enzyme Titration.** Data are shown as relative light units (RLU) that directly correlate to the amount of ADP produced. The correlation between the % of ATP converted to ADP and corresponding signal to background ratio is indicated for each kinase amount.

| MSK1, ng | 200 | 100 | 50 | 25 | 12.5 | 6.3 | 3.1 | 1.6 | 0.8 | 0.4 | 0.2 | 0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Luminescence | 48036 | 40882 | 38352 | 28608 | 17047 | 8512 | 4101 | 1859 | 963 | 516 | 329 | 154 |
| S/B | 312 | 265 | 249 | 186 | 111 | 55 | 27 | 12 | 6 | 3 | 2 | 1 |
| % Conversion | 56 | 48 | 46 | 36 | 20 | 10 | 4 | 2 | 1 | 0.3 | 0.1 | 0 |



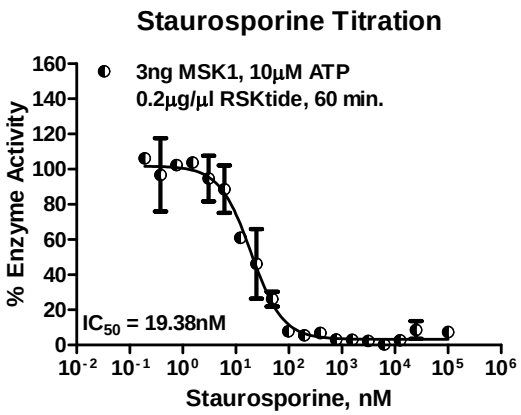




**Figure 3. MSK1 Kinase Assay Development.** **(A) MSK1 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Staurosporine dose response was created using 3ng of MSK1 to determine the potency of the inhibitor ($IC_{50}$).**

**Assay Components and Ordering Information:**

| Products | Company | Cat.# |
|---|---|---|
| ADP-Glo™ Kinase Assay | Promega | V9101 |
| MSK1 Kinase Enzyme System | Promega | V5092 |
| ADP-Glo™ + MSK1 Kinase Enzyme System | Promega | V5093 |

MSK1 Kinase Buffer: 40mM Tris, pH 7.5; 20mM $MgCl_2$; 0.1mg/ml BSA; 50µM DTT.